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The Chemical Synthesis of Peptides

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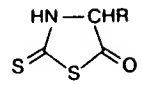
Balliol College, Oxford J.H.J.
May 1990



(25).NTA

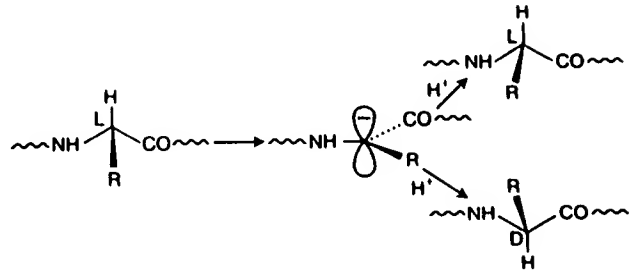
case), and the progressive accumulation of over-reaction products limits the extent to which repetitive approach can be taken without difficult purification problems. The thio-analogues or NTAs (**25**) have also been used for peptide synthesis.[65] They are less prone to yield over-reaction products than NCAs, as the thiocarbamic acids produced by their aminolysis are less fragile. They are preferred in the special cases of glycine and histidine (the NCAs of which are especially subject to troublesome side-reactions), but unfortunately they are not secure against racemization.

*5.1.2 Racemization**

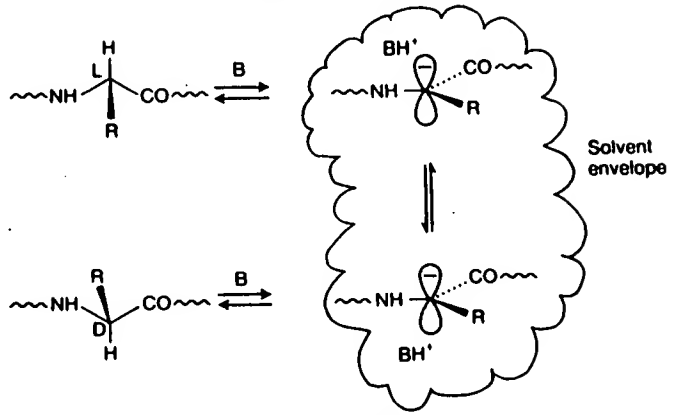
Consider the synthesis of an all-L peptide comprising n chiral residues, from optically pure α-amino acids. If the operations needed for the incorporation of each residue result in conversion of a small fraction f of each residue to the D-form, and the epimers are carried through without separation, then the end product will consist of the required all-L peptide and a blend of other peptides in approximate proportions $(1\text{-}fn):fn$. For a synthesis of a 50-residue peptide in which 1% D-residue formation takes place at each stage, only half the final product will have the required all-L stereochemistry. The other 50% will consist mainly of about 1% each of all the 50 possible epimers with one D-residue. This will in general pose a prohibitive purification problem, and racemization in peptide synthesis has therefore been closely studied, with a view to defining the conditions under which it is minimal.[66–68]

Except for special cases (e.g., synthesis with *N*-methylamino acids: see Section 6.2.1), racemization is an almost exclusively base-induced side-reaction, and in practice is only a matter for serious concern at the activation and coupling stages of a synthesis. There are two important mechanisms.

*This term is used in peptide chemistry in a loose way which not only covers the strict sense as defined in most general organic chemistry texts (conversion of an enantiomer to a mixture of enantiomers), but also embraces partial epimerisation, whereby there is loss of chiral integrity at one out of two or more chiral centres, resulting in the formation of a mixture of epimers (i.e. diastereoisomers differing at one chiral centre).



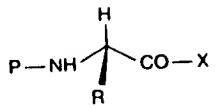
Scheme 5.30.



Scheme 5.31.

5.1.2.1 Direct enolization

Deprotonation at the α-carbon of an α-amino-acid residue results in racemization, because the carbanion intermediate can reprotonate on either side (Scheme 5.30). This has been called the 'direct exchange' mechanism, an inappropriate expression, because under some circumstances[69] racemization is much faster than exchange with the proton pool, implying that an ion pair is formed in which the ions are jostled about by solvent molecules and change their relative orientations without being divorced from each other, so that reprotonation can return the original proton to either side of the chiral centre ('isoracemization': Scheme 5.31). The rate of racemization by direct enolization depends on the catalysing base, the solvent, and the electron-withdrawing effects of the groups P,R, and X around the chiral centre (**26**). When X=NH-, O-alkyl, or O^-, it is in most cases negligible,

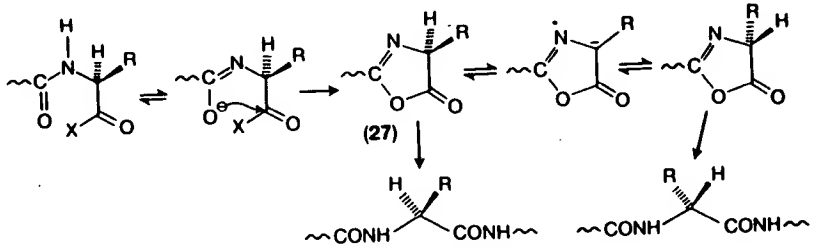


(26)

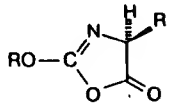
and basic deprotection procedures other than saponification (see Section 4.1.1) are generally completely safe. During activation and coupling, the risk is rather more significant, but the danger is over once coupling is complete. Racemization is fastest with strongly electron-withdrawing groups X (i.e. most good leaving groups), and unhindered strong bases in dipolar aprotic solvents like DMSO and DMF. Inessential exposure to strong bases is clearly to be avoided, but the best way of responding to the other factors is not so obvious, because what matters is not the rate of racemization *per se*, but the balance between the rates of racemization and coupling, and this is much more difficult to make reliable generalizations about. Fortunately, with the exception of (a) a few special amino acids (α-arylglycines present quite a serious problem), and (b) couplings which are inordinately slow, the amount of racemization which actually takes place by this pathway is very slight indeed.

5.1.2.2 The oxazolone mechanism

Activated acylamino acids and peptides cyclize under the influence of base to give oxazolones [**27**; strictly '5(4*H*)-oxazolones', formerly '2-oxazolin-5-ones', archaically 'azlactones']. The oxazolones so formed are themselves activated towards aminolysis, and reaction with amino components leads ultimately to peptides, but since their racemization via stabilized anions is usually fast compared to the rate of peptide bond formation, any peptide thus produced is largely racemized (Scheme 5.32). Oxazolones are actually useful (e.g., references 70 and 71; see Scheme 6.31) for the activation of α-dialkyl-α-amino acid residues, where the question of racemization by base



Scheme 5.32. Conditions: basic.



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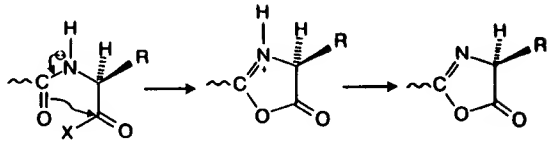
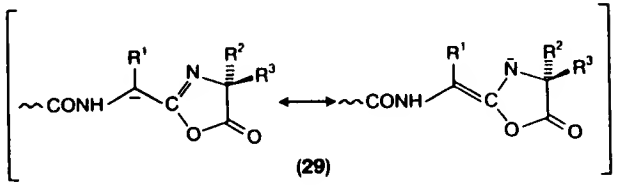


Scheme 5.33.

does not arise because there is no hydrogen at the α-carbon. When the amino-nitrogen of the activated residue is acylated with a simple acyl group (acetyl, benzoyl, etc.), or with a peptide chain, cyclization to the oxazolone occurs easily with most good leaving groups X, and gross or even complete racemization may ensue. But oxazolone formation is not so facile when the acyl substituent is an alkoxycarbonyl protecting group. Indeed, the process was held to be impossible until 1977.[72] Furthermore, the alkoxyoxazolones **28** are both less easily racemized and more easily aminolysed than are the oxazolones **27** derived from simple acylamino acids. The activation of ordinary Z, Boc and Fmoc amino acids, etc., and their coupling with amino components is consequently not attended by the danger of racemization under normal conditions. This is a pivotal fact on which much of modern peptide synthesis turns. The reason for the contrast between, e.g., Z and benzoyl amino acids has not been fully explained, but a major factor is probably the lower acidity of BzlOCONH- compared to PhCONH-. In Scheme 5.32, the ring closure is shown as a specific base catalysed process, which has been demonstrated to be so in one set of circumstances.[72] It might be a concerted general base catalysed process under other conditions, but in either case lowering the acidity of the NH would be expected to diminish the rate of oxazolone formation.

A mechanism for oxazolone formation which does not require base assistance is also possible (Scheme 5.33), but ordinary amino acid derivatives do not cyclize this way except under very vigorous activation. *N*-Methyl-α-amino acid derivatives do, however, and give optically labile oxazolonium cations (see Scheme 6.29) even under normal activation conditions:[74] base catalysis is impossible because there is no NH for it to operate through, so cyclization can only occur by attack of the neutral amide oxygen on the activated carbonyl, which is easier with -CONMe- than -CONH- because



(29)

of electron release by the methyl group. Conformational restraints fortunately prevent this happening with proline derivatives, under all except the most extreme conditions.

Whether or not oxazolone-mediated racemization accompanies the activation and coupling of susceptible protected peptide acids depends on the leaving group. It does not seem to do so to a significant extent with acyl azide intermediates, in model systems at least, perhaps because these owe their aminolytic reactivity to intramolecular general base catalysis (see Section 5.1.1.2), to which oxazolone formation is indifferent because the nucleophile bears no hydrogen. And the risk is also small with the DCCI-HOBt procedure (see Section 5.1.1.4), because HOBt rapidly intercepts the activated species which might otherwise degenerate into oxazolones. The reactive HOBt ester intermediate favours aminolysis over oxazolone formation, possibly in part for the same reason as suggested for azides.

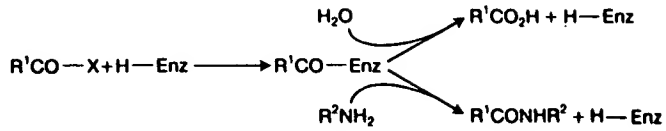
With activated protected peptides, the direct enolization and oxazolone mechanisms both provide pathways for the racemization of the carboxy-terminal residue. The formation of an oxazolone also threatens the chiral integrity of the penultimate residue, because the carbanion **29** is stabilised. Racemization at that residue has been observed at moderate levels in model experiments (e.g., reference 75), but has not so far been recognized as a real problem in actual syntheses, except when deliberate oxazolone formation is used to drive the coupling of carboxy components terminating in -XaaAibOH.[71]

5.2 The use of enzymes

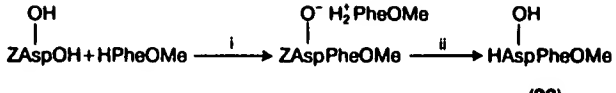
This book is concerned with the chemical synthesis of peptides, so the inclusion of enzymic methods may raise a few eyebrows, but the use of enzymes as reagents in preparative organic chemistry,[76] without special homage to their biological origin, is burgeoning. A few remarks on enzymatic peptide bond formation[77,78] therefore seem called for.

Nature provides a wide range of proteolytic enzymes which can in principle be perverted to catalyse peptide bond formation by manipulating the conditions. There are two strategies for doing this. The first is dependent on thermodynamic control, the equilibrium in Scheme 5.34 (which

Scheme 5.34.



Scheme 5.35.



(30)
Aspartame

Scheme 5.36. Conditions: i, 2 equiv. HPheOMe/pH7/thermolysin (the dipeptide salt precipitates); ii, HCl, then catalytic transfer hydrogenolysis with HCO_2NH_4/Pd(C)/MeOH.

favours hydrolysis overwhelmingly under normal conditions) being somehow displaced in favour of peptide bond formation. This can be achieved by employing protecting groups which will ensure precipitation of the peptide, or by using biphasic systems so that the peptide passes out of the aqueous phase into an organic solvent as it is formed, or by using water-miscible organic solvents which perturb the dissociation constants of the components and shift the balance of the equilibrium. The second strategy exerts kinetic control by arranging for an amino component nucleophile to compete with water for an acyl-enzyme intermediate (Scheme 5.35). The advantages of an enzymatic synthesis are the mild conditions, freedom from racemization and the need for side-chain protection, the possibility of using immobilized enzyme technology[79,80] with catalyst recovery, and the scope for industrial scale-up. Many examples have been reported. The synthesis[81] of the synthetic sweetener aspartame (**30**) is one of particular interest which has been developed for commercial application, and is also simple enough to be an undergraduate exercise[82] (Scheme 5.36). There are disadvantages, however. With peptides longer than dipeptides, there is the danger that while the protease is being persuaded to work backwards in creating a peptide bond at one point, it will remember the purpose for which evolution devised it and dismantle another somewhere else. No new case can be treated as

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